NUCLEAR SCIENCE AND TECHNIQUES **25**, 040201 (2014)

External micro-PIXE analysis of Nd^{3+} accumulation in Euglena gracilis*

LI Xin-Yi (李忻忆),[1] Satoh Takahiro,[2] Yasuyuki Ishii,[2] Takeru Ohkubo,[2] LI Yong-Qiang (李永强),[3,4] REN Qing-Guang (任庆广),[5,†] RONG Cai-Cai (荣彩彩),[1] LÜ Hao-Yan (吕浩岩),[1] and SHEN Hao (沈皓)[1,‡]

[1]*Institute of Modern Physics, Applied Ion Beam Physics Laboratory, Fudan University, Shanghai 200433, China*
[2]*Takasaki Advanced Radiation Research Institute, Japan Atomic Energy Agency, 1233 Watanuki-machi, Takasaki, Gunma 370-1292, Japan*
[3]*Department of Radiation Oncology, Fudan University Shanghai Cancer Center, Shanghai 200032, China*
[4]*Department of Oncology, Shanghai Medical College, Fudan University, Shanghai 200032, China*
[5]*Research Center for Analysis and Measurement, Fudan University, Shanghai 200433, China*

(Received January 23, 2014; accepted in revised form March 25, 2014; published online July 26, 2014)

External micro-PIXE measurements were done to investigate the accumulation of Nd^{3+} on green algae species euglena gracilis. According to the Nd distribution patterns in the gracilis cells, the biosorption of Nd^{3+} to the cell's compartments can be observed. Comparing elemental mappings of the cell treated with different doses of the 1 mg/mL Nd^{3+} solution, the Nd uptake of euglena gracilis cells do no relate with the doses. From distributions of Ca and Mg, it is found that the Ca is complementary to Nd partly, and the Nd and Mg distributions are alike to each other, showing that Nd may be mainly in the chlorophyll molecules. The biochemistry related is discussed.

Keywords: Euglena gracilis cells, External micro-PIXE, Nd^{3+} accumulation

DOI: 10.13538/j.1001-8042/nst.25.040201

I. INTRODUCTION

Biosorption of microorganisms, such as bacteria, fungi and algae, is a good means to accumulate heavy metal ions from water [1, 2]. Euglena gracilis, a unicellular green alga, demonstrates a remarkable ability to accumulate rare earth element (REE) cations to cell compartments. It was found that such cells could transport REE cations Nd^{3+} actively against apparent gradient in the order of 5 or higher [3]. Apart from its intrinsic interest, algal cells exhibiting this trait can be of value in application to biological elimination in aquatic environment for removing both toxic metal ions and organic contaminants. It is known that the cellular effects of lanthanide, including cell fusion, muscle relaxation, blood coagulation, etc., are quite diverse [4]. Since the last decade, the bio-medical aspects and toxicity of REE ions have been comprehensively reviewed [5–7]. However, very little is known about the mechanism of the REE ion uptake process, intracellular transport and storage of REEs in cells.

Micro-PIXE has been successfully applied to many biological, medical and environmental problems with its high sensitivity and good spatial resolution. Additionally, using an external beam has many advantages, requiring no special sample preparations against vacuum environment, easier handling and observing the samples, reducing damage by heating and charging [8]. In this paper, we study bioaccumulation of the REE cations Nd^{3+} by measuring its distribution patterns in Euglena gracilis with external micro-PIXE, towards a better understanding of the accumulation process and its consequences for other ions.

* Supported by the National Natural Science Foundation of China (Nos. 10675033 and 10975034)
† qgren@fudan.edu.cn
‡ haoshen@fudan.edu.cn

II. EXPERIMENTAL

A. Cell culture and sample preparation

Euglena gracilis 848 was obtained from the Institute of Hydrobiology (Wuhan), Chinese Academy of Sciences. Details for the cell culture were described in Ref. [9]. The cells were centrifuged by three cycles of re-suspension in de-ionized water to remove culture medium. The euglena 848 cell suspension (3 mL) were syncretized with 1 mL or 0.1 mL of 1 mg/mL $NdCl_3$ solutions. The cells, now loaded with metal ions, were further washed by three cycles of re-suspension. Then, the cells were fixed by 0.25% glutaraldehyde ($CHO(CH_2)_3CHO$). The cell suspension in de-ionized water was dropped onto Mylar film with low cell density for single cell external micro-PIXE measurements.

B. External micro-PIXE

The external micro-PIXE experiments were carried out using the 3 MV single-ended accelerator generating 3.0 MeV proton beams on TIARA (Takasaki Ion Accelerators for Advanced Radiation Application) [8, 10]. A 2-μm-thick Mylar foil was used for the exit window to withstand the pressure differential. The cells were attached on the Mylar foil directly, so there is no air from target to the detector. The final resolution of the proton beam was 1.2 μm. A Si(Li) X-ray detector (30 mm^2 active area, energy resolution of 135 eV) was placed in a vacuum chamber, so as to avoid interference from argon K X-rays, and located at 140° with respect to the beam incidence. The detector window is 8 μm beryllium. An annular type absorber (100-μm Mylar, with a Φ3 mm hole) was used, for detecting Mg and P. DAQ software was capable of simultaneous processing signals from the detector. The

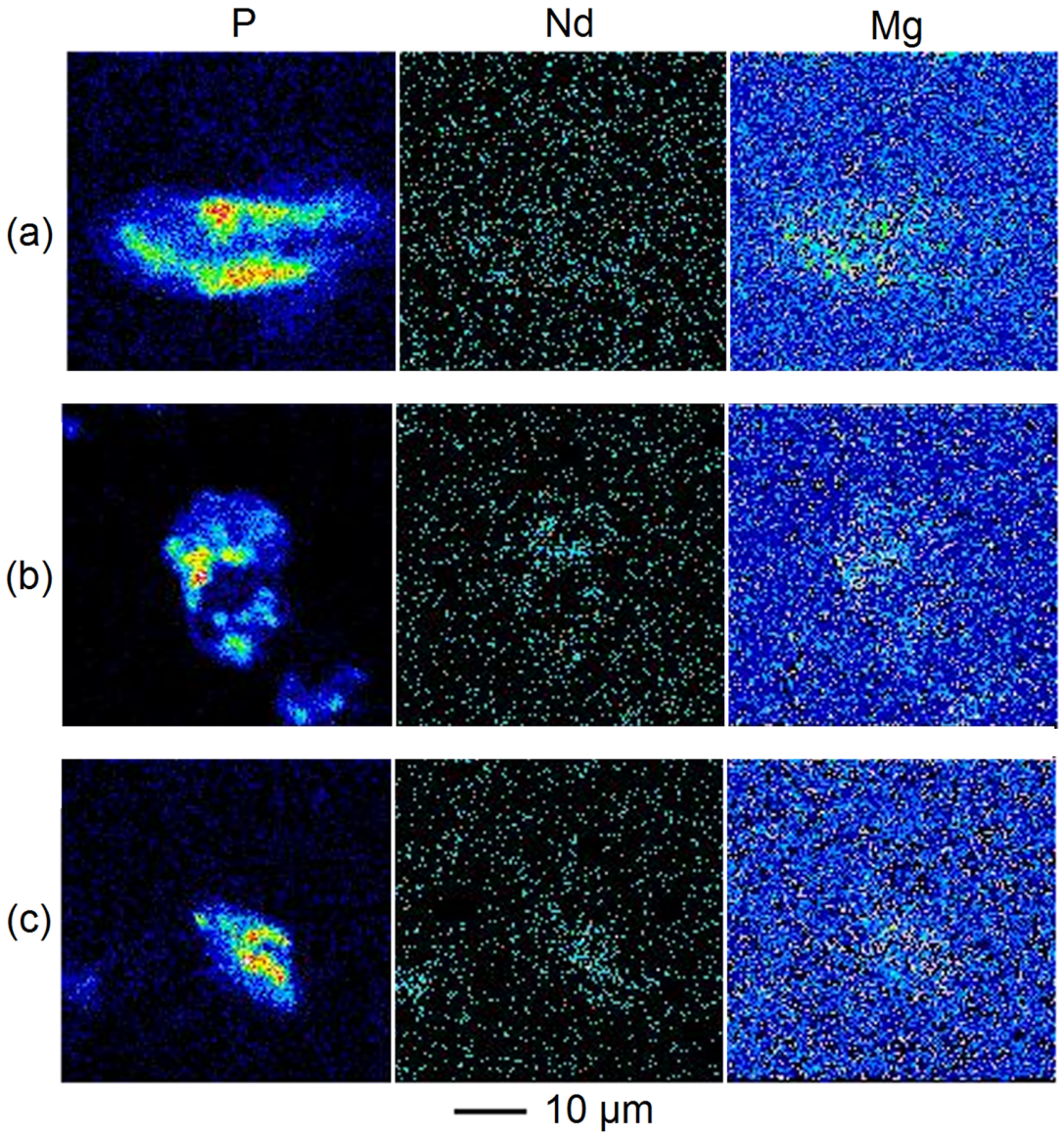

Fig. 1. (Color online) Distributions of P, Nd and Mg in a single gracilis cell treated with different doses of 1 mg/mL $NdCl_3$ solutions. (a) 0 mL, (b) 1 mL, (c) 0.1 mL. The Nd and Mg distribution patterns are alike each other, showing Nd enrichment in chloroplasts. The scan size is 50 μm × 50 μm.

beam current was 100–200 pA. The cells were scanned with integrating charge of 130–200 nC to obtain matrix and trace element distribution.

III. RESULTS AND DISCUSSION

PIXE spectra of the cells were extracted from the raw scanning data according to their shapes. The intensity of neodymium L-X-rays was used to produce elemental mapping of the cells [1]. Fig. 1 shows elements distributions in gracilis cells treated with different doses of $NdCl_3$ solutions, with (a) being the control, and (b) and (c) being a cell treated with 1 mL and 0.1 mL of 1 mg/mL $NdCl_3$ solutions, respectively. The cells in each group were randomly selected.

The distribution of phosphorus fit almost with the cell shape observed under a microscope, as phosphorus is the domain element of cell membrane. For Nd distribution, compared with group (a) and (b), there is no obvious local enrichment of Nd in the group of 1 mL $NdCl_3$ (1 mg/mL), while in the group of 0.1 mL $NdCl_3$ (1 mg/mL), the Nd distributed over the cell compartments. Evidently, Nd^{3+} had been transported inside the cells. It is in accordance with our previous work done in Centre for Ion Beam Applications of National University of Singapore [11, 12]. Additionally, the results of cryosections of I4TCF-Nd^{3+} stained cells and EDAX of fast freezing ultrathin cyosections, and as the experiments of electron microscopy on the alga [13], showed the same conclu-

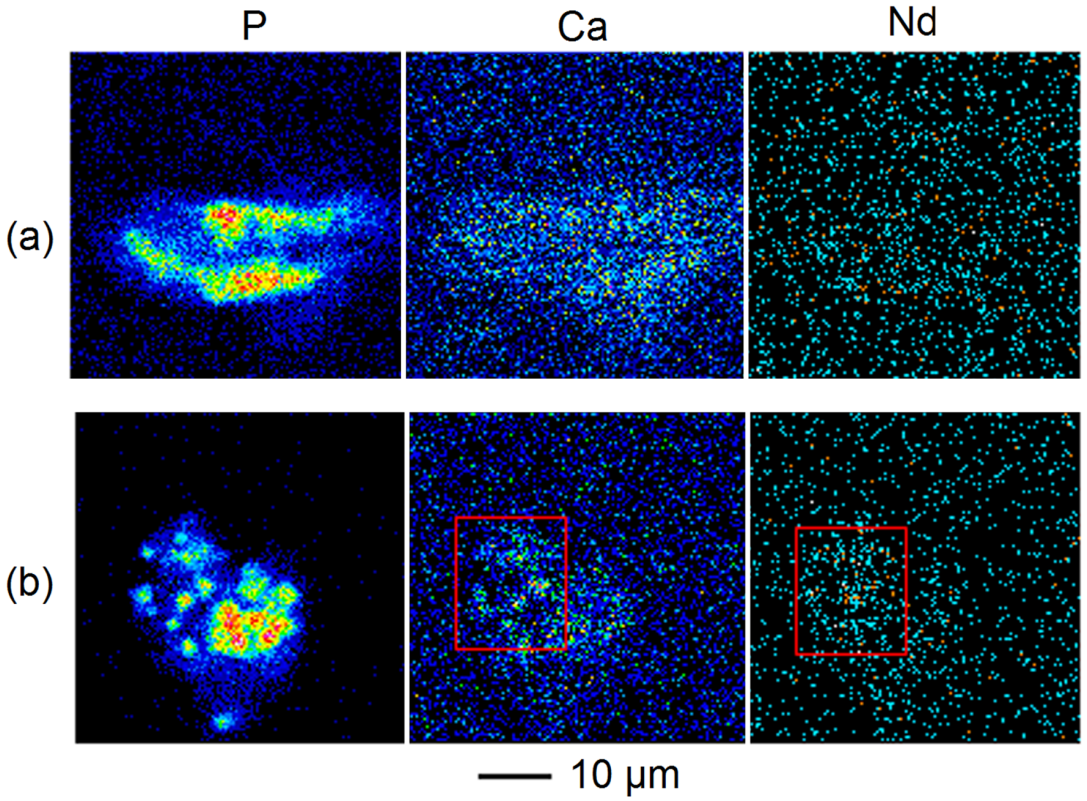

Fig. 2. (Color online) Element mapping of a single gracilis cell in a 50 μm × 50 μm scan area. (a) the control; (b) treated with 1 mL of 1 mg/mL $NdCl_3$ solutions. Red rectangles show that Ca is complementary to Nd partly in the cell loaded with Nd, while this phenomenon could not be seen markedly in the control sample.

sion. Lansman [14] suggested that lanthanide ions were able to traverse the channel and enter the cell interior. Each alga cell is equipped with a single transport apparatus [15, 16], which enables the Nd^{3+} uptake. This shares some characteristic with calcium ion channels [13]. Comparing Figs. 1(b) and 1(c), with the cells having been treated with different doses of 1 mg/mL Nd^{3+} solution, the cellular uptake of Nd^{3+} is independent of Nd^{3+} concentration in the bulk solution, which had been proved by ICP-AES [9, 13].

It is known that chloroplasts are the major compartments as the residences of Nd [13]. In nature, magnesium is the coordination center of chlorophyll. Lanthanide-substituted chlorophyll has been investigated [17, 18]. EXAFS (extended X-ray absorption fine structures) results suggest that exogenous lanthanide might substitute for the magnesium in chlorophyll after it is transported to the interior of algal cells [19]. Energy-dispersive X-ray microanalysis (EDXA) was used to investigate chloroplasts. A characteristic peak of lanthanide in chloroplasts appeared clearly [13]. Also, in Figs. 1(b) and 1(c), the Nd and Mg distribution are of similar patterns. This provides again good evidence that loaded neodymium may couple with chlorophyll. In addition, comparing with the control, the Mg content in the treated cells deceased approximately by 70%, in both Figs. 1(b) and 1(c), showing that Nd substitutes for Mg in chlorophyll after it is transported into the cell.

Figure 2 shows Ca and Nd distributions in a single cell from the control and the Nd^{3+}-treated cells. Although the Ca and Nd distributions differ from each other, it can be found (see the red rectangle) that calcium is complementary to neodymium partly in the cell loaded with Nd, while this phenomenon cannot be seen markedly in the control sample. Probably, this indicates that the Ca content decreased after uptake of the Nd cations. The distribution radius of the rare earth ions is very close to that of Ca^{2+}; they can bind with some bio-macromolecules to form coordination compounds and replace Ca^{2+} on protein in binding sites [4, 20].

IV. CONCLUSION

Nd^{3+} accumulation in euglena 848 cells is revealed by external micro-PIXE analysis, and the relationships of the Nd distribution pattern with that of Ca and Mg are investigated. The neodymium ions traverse the channel and enter into the cell. Probably, they replace calcium ions on protein in binding sites, and the magnesium ions in chlorophyll, being enriched in chloroplasts. A deeper understanding of the Nd transport mechanism requires further investigation.

[1] Guo P, Wang J, Li X, *et al*. Nucl Instrum Meth B, 2000, **161–163**: 801–807.
[2] Wang H K and Wood J M. Environ Sci Technol, 1984, **18**: 106–109.
[3] Kang L, Shen Z Q, Jin C Z. Chinese Sci Bull, 2000, **45**: 585–591.
[4] Shen H, Ren Q G, Mi Y, *et al*. Nucl Instrum Meth B, 2002, **189**: 506–510.
[5] Hirano S and Suzuki K T. Exposure metabolism and toxicity of rare earths and related compounds. Environ Health Perspect. 1996, **104** (suppl 1): 85–95.
[6] Ni Z J. Bioinorganic Chemistry of Rare Earth Elements. Beijing (China): Science Press, 1995.
[7] Brown P H and Rathjen A H, *et al*. Handbook of Physics and Chemistry of Rare Earths. Amsterdam (Netherlands): Elsevier Science Publishers, 1990.
[8] Sakai T, Kamiya T, Oikawa M, *et al*. Nucl Instrum Meth B, 2002, **190**: 271–275.
[9] Jin C Z, Kang L, Shen Z Q, *et al*. Acad Period Abstr China, 1998, **4**: 976–980. (in Chinese)
[10] Sakai T, Oikawa M, Sato T, *et al*. Nucl Instrum Meth B, 2005, **231**: 112–116.
[11] Zheng Y. Ph.D. Thesis, Fudan University, 2012. (in Chinese)
[12] Ren M Q, Chen X, Zheng Y, *et al*. X-Ray Spectrom, 2013, **42**: 237–241.
[13] Kang L, Shen Z Q, Jin C J. Chinese Sci Bull, 2000, **45**: 585–591.
[14] Lasman J B. J Gen Physiol, 1990, **95**: 679–696.
[15] Wolken J J. Euglena: An Experimental Organism for Biochemical and Biophysical Studies, ed. 2. New York (USA): Appleton-Century-Crofts, 1967.
[16] Miller R J. Science, 1987, **235**: 46–52.
[17] Wu Z Y, Tao Y, Benfatto M, *et al*. J Synchrotron Radiat, 2005, **12**: 98–101.
[18] Hong F S, Wei Z G, Zhao G W. Sci China Ser C, 2001, **31**: 392–400. (in Chinese)
[19] Ren Q G, Hua Y, Shen H, *et al*. J Radioanal Nucl Ch, 2007, **272**: 359–362.
[20] Wang K. Trace Elements in Life Science. Beijing (China): Chinese Measuring Press, 1992. (in Chinese)